Taxonomy vs Phylogeny

Taxonomy was the traditional way that biologists recognised individual species (Genus species binomial).

It was the tool that allowed identification of species in a broader hierarchy

- Family, Order, Class, Phylum, Kingdom, etc.

It was generally considered to reflect the phylogenetic history of species.

- But it didn't really matter if it didn't ….. it was a tool to identify species.

Phylogeny

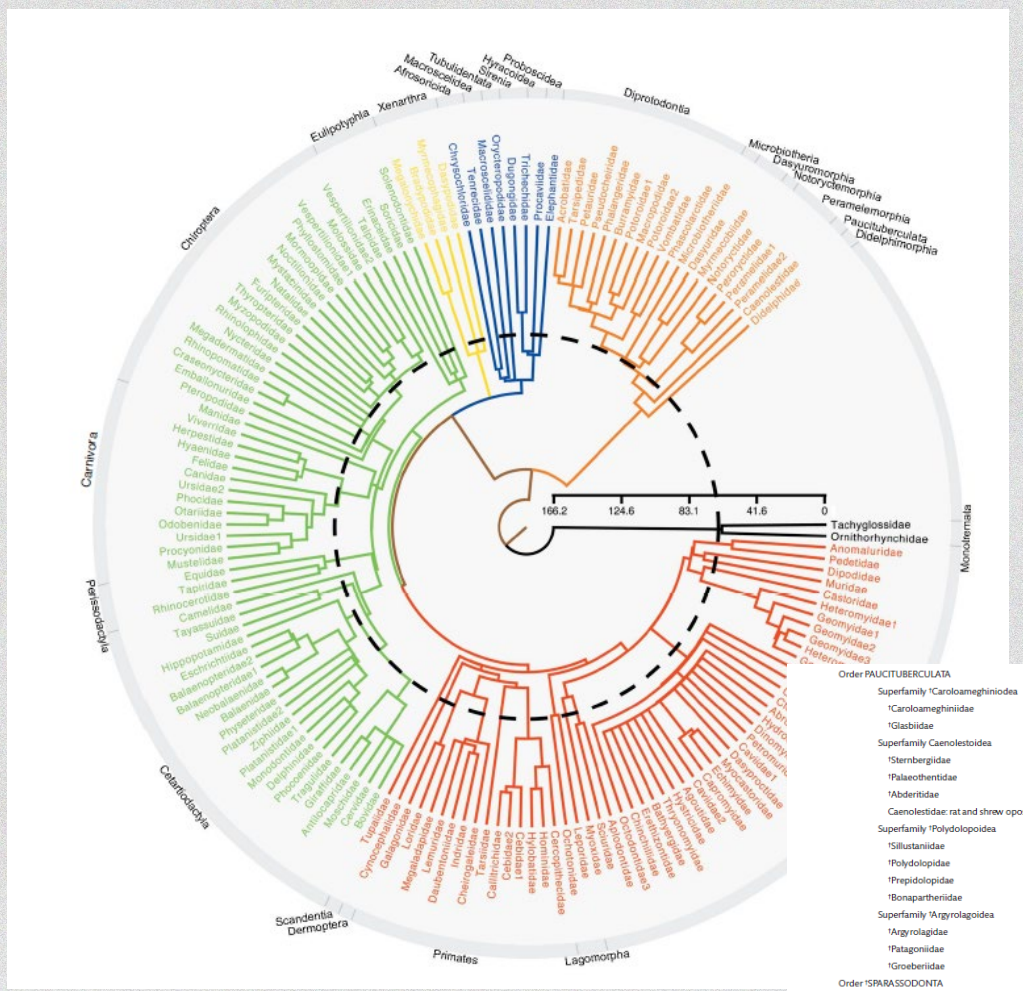
Phylogeny is the actual evolutionary relationship of species.

A phylogeny has more information (detail) of relationships than taxonomy.

A phylogeny was traditionally based on morphology, embryology, even physiology.

Now advances in molecular biology (gene sequencing) have enormously contributed to “modern” phylogenies.

We can NEVER know the REAL phylogeny – we can only infer (“educated guess”) it!



COMPARING SPECIES:

Phylogeny and Taxonomy
provide a framework to compare species

The goal is to understand the selective pressures that have led to animal traits. How do we know?

Convergence (of dissimilar organisms to a common environment)

Divergence (of similar organisms into new environments)

Relatedness (all organisms are phylogenetically related)

Comparative Animal Physiology

Comparative animal physiology, defined in a broad context, is the comparing and contrasting of physiological mechanisms, processes, or responses of different species of animals, or of a single species, under differing conditions. The most interesting comparisons and contrasts are often the physiological convergences observed for distantly related animals adapted to similar environments (e.g., desert-adapted insects and mammals) because this illustrates the independent evolution of solutions to common problems, or the physiological divergence observed for closely related animals adapted to different environments (e.g., fresh water, brackish water, and seawater teleost fish) because this illustrates the modification of originally similar physiological processes to cope with different problems, or for the physiological responses of individual animals under differing conditions (e.g., at different temperatures) because this illustrates the intraindividual plasticity of an animal's physiology. Physiological adaptation is often most evident at high taxonomic levels (e.g., comparing classes, orders, families) but is also evident at lower levels (genus, species, and even subspecies).

How do we compare species?
It is easy to do a 2-species comparison – is it wrong? No!

The standard approach to doing a two-species comparison is to do a t-test:

In comparing two birds, a desert and a mesic species, the means of a trait for the two species can be tested to see if they are significantly different.

There are also various non-parametric tests for two group comparisons.

Here, there is a difference between the two species.

desert	mesic
8.8	9.9
8.4	9.0
7.9	11.1
8.7	9.6
9.1	8.7
9.6	10.4
	9.5

t-Test Result for Datasets:
Set 1 Range = Sheet1!$A$1:$A$7
Set 2 Range = Sheet1!$B$1:$B$8

Descriptive Statistics

Variable	Mean	Std Dev.	Std Err	Lower 95% CL	Upper 95% CL	N
desert	8.750	0.582	0.238	8.139	9.361	6
mesic	9.743	0.818	0.309	8.986	10.500	7

F-Test for Equality of Variances

Variable	Variance	F	DF 1	DF 2	P
desert	0.339	1.975	6	5	0.236
mesic	0.670				

Sample variances don't differ at the specified alpha of 0.0500
so the pooled variance of 0.5193 is used in the t-Test.

2-tailed t-Test

Ho. Diff	Mean Diff.	SE Diff.	T	DF	P
0.000	-0.993	0.401	-2.476	11.000	0.031

But what was the question?

Is the question:
Do the two species differ? ✓ (differ in the trait we measured)

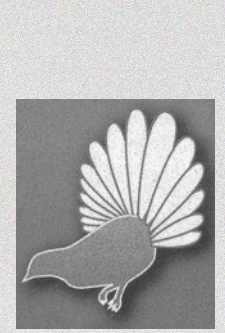
or is it?:
Does the difference provide statistical evidence of a link between the trait in question and the type of environment? ✗

or is it?:
Does the study suggest that the observed differences are consistent with the hypothesis, which can be compared with the results of similar studies? ✓

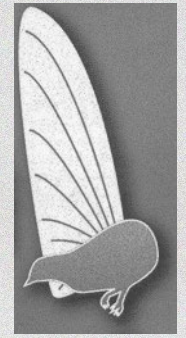
So what is the problem with 2-species comparisons?

The problem is intent/statistical: We are not isolating the treatment (environmental water levels) we are interested in. Any differences we find could be attributed to any difference between the species or their environment.

- Replication? Control? Randomisation? (how could these be used to improve the situation?



compare



desert
tropical
high-altitude
passerine

non-desert
non-tropical
low-altitude
non-passerine

etc

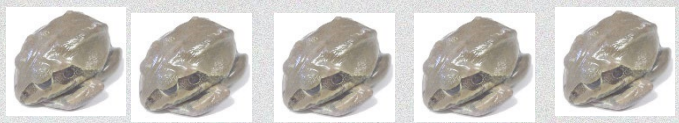
Are you convinced this is a desert adaptation?

Consider this example: Is cocoon formation related an adaptation to living in the desert?

You decide to measure cocoon formation for 5 desert and 5 mesic frog species; You hypothesise cocooning (√) to be a desert adaptation and non-cocooning (x) to be a mesic adaptation

You find: Desert species Mesic species

√ √ √ √ √ x x x x x



Simple statistics: What is the change that all 10 predictions come out as expected? → P=0.5^10=0.000977 (one-tailed) or P=0.5^9=0.00195 (two-tailed)

Are you still convinced this is an adaptation?

You realise that:

- all of the desert species are Neobatrachus
- all of the mesic species are Heleioporus

Neobatrachus species	Heleioporus species
√ √ √ √ √	x x x x x

Simple statistics: What is the change that all 10 predictions come out as expected? →
P=0.5^2=0.25 (one-tailed) or P=0.5^1=0.5 (two-tailed) !!

The problem with relatedness of species….

Are these 5 species of Neobatrachus independent of each other?

Are these 5 species of Heleioporus independent of each other?

No! Closely-related species are likely to be less independent than distantly-related species.

Species are NOT independent data points

Conventional Statistics: ANOVA and REGRESSION

There are two **conventional strategies** that are often used for analysing multiple species data:

- analysis of variance (ANOVA)
- linear regression (OLSR)

Which one we use depends on whether we have measured one or two numerical traits:

- if we measure **one numerical** trait (*e.g.* metabolic rate), and **one categorical** factor (e.g. arid, mesic, polar), then we analyse the data by **ANOVA**.
- If we measure **two numerical** traits (e.g. metabolic rate and body mass), then we analyse the data by **linear regression**.

But these ignore relatedness, or phylogeny! – Just like the simple stats I showed earlier.

One trait – ANOVA single factor (species)

species	metabolic rate
desert A	28.2
desert A	33.2
desert A	36.4
desert A	34.6
desert A	29.1
desert A	31
desert B	39.6
desert B	40.8
desert B	37.9
desert B	37.1
desert B	43.6
desert B	42.4
mesic C	46.3
mesic C	42.1
mesic C	43.5
mesic C	48.8
mesic C	43.7
mesic C	40.1
mesic D	41
mesic D	44.1
mesic D	46.4
mesic D	40.2
mesic D	38.6

Analysis of Variance results for:
Y Variable Range = $B$1:$B$24
Factor Range = $A$1:$A$24

Descriptive Statistics

Group	Mean	Std Dev.	Std Err	N
desert A	32.083	3.205	1.309	6
desert B	40.233	2.530	1.033	6
mesic C	44.083	3.081	1.258	6
mesic D	42.060	3.145	1.406	5

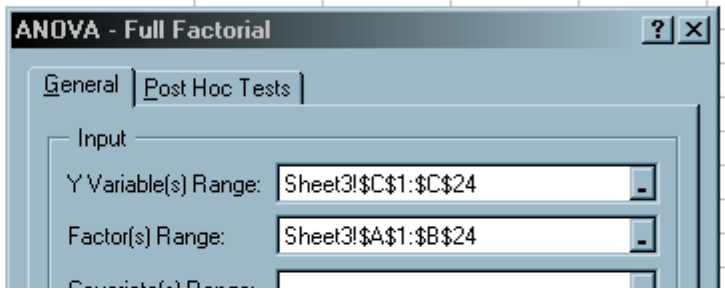
Analysis of Variance for Y=metabolic rate

Source	Type III SS	Df	Mean Sq.	F	Prob.
Model	492.076	3	164.025	18.291	0.000
Error	170.382	19	8.967		
Total	662.458	22			

There is a significant effect of species on metabolic rate – but what about **desert** versus **mesic** differences?

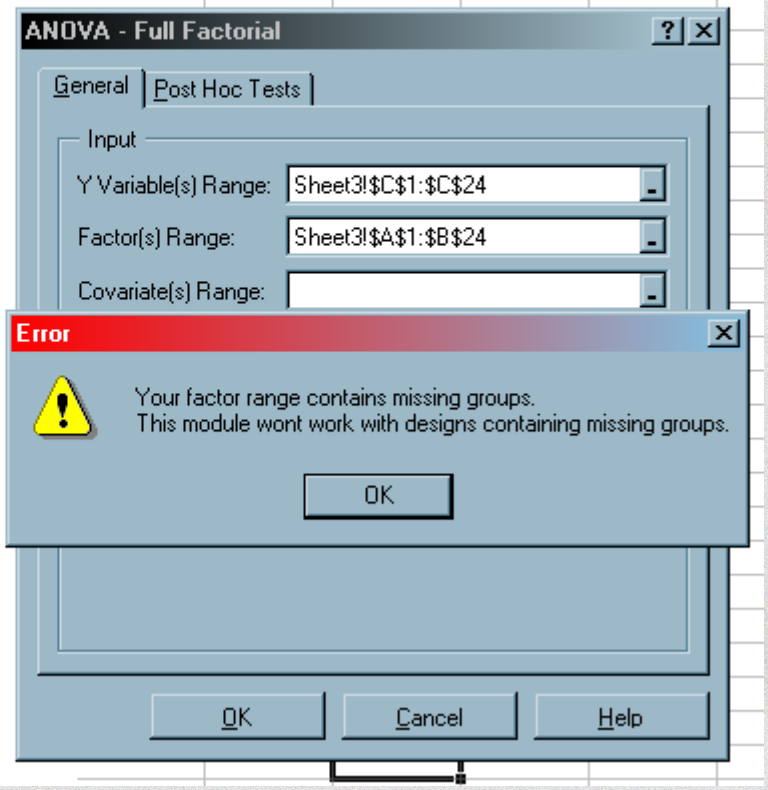
two factor ANOVA (species and habitat)

	A	B	C
1	habitat	species	metabolic rate
2	desert	A	28.2
3	desert	A	33.2
4	desert	A	36.4
5	desert	A	34.6
6	desert	A	29.1
7	desert	A	31
8	desert	B	39.6
9	desert	B	40.8
10	desert	B	37.9
11	desert	B	37.1
12	desert	B	43.6
13	desert	B	42.4
14	mesic	C	46.3
15	mesic	C	42.1
16	mesic	C	43.5
17	mesic	C	48.8
18	mesic	C	43.7
19	mesic	C	40.1
20	mesic	D	41
21	mesic	D	44.1
22	mesic	D	46.4
23	mesic	D	40.2
24	mesic	D	38.6



It doesn't work!

	A	B	C
1	habitat	species	metabolic rate
2	desert	A	28.2
3	desert	A	33.2
4	desert	A	36.4
5	desert	A	34.6
6	desert	A	29.1
7	desert	A	31
8	desert	B	39.6
9	desert	B	40.8
10	desert	B	37.9
11	desert	B	37.1
12	desert	B	43.6
13	desert	B	42.4
14	mesic	C	46.3
15	mesic	C	42.1
16	mesic	C	43.5
17	mesic	C	48.8
18	mesic	C	43.7
19	mesic	C	40.1
20	mesic	D	41
21	mesic	D	44.1
22	mesic	D	46.4
23	mesic	D	40.2
24	mesic	D	38.6



Not all combinations of the two factors are present
e.g. species A is only desert

How do we compare multiple species by REGRESSION?

Two numeric traits

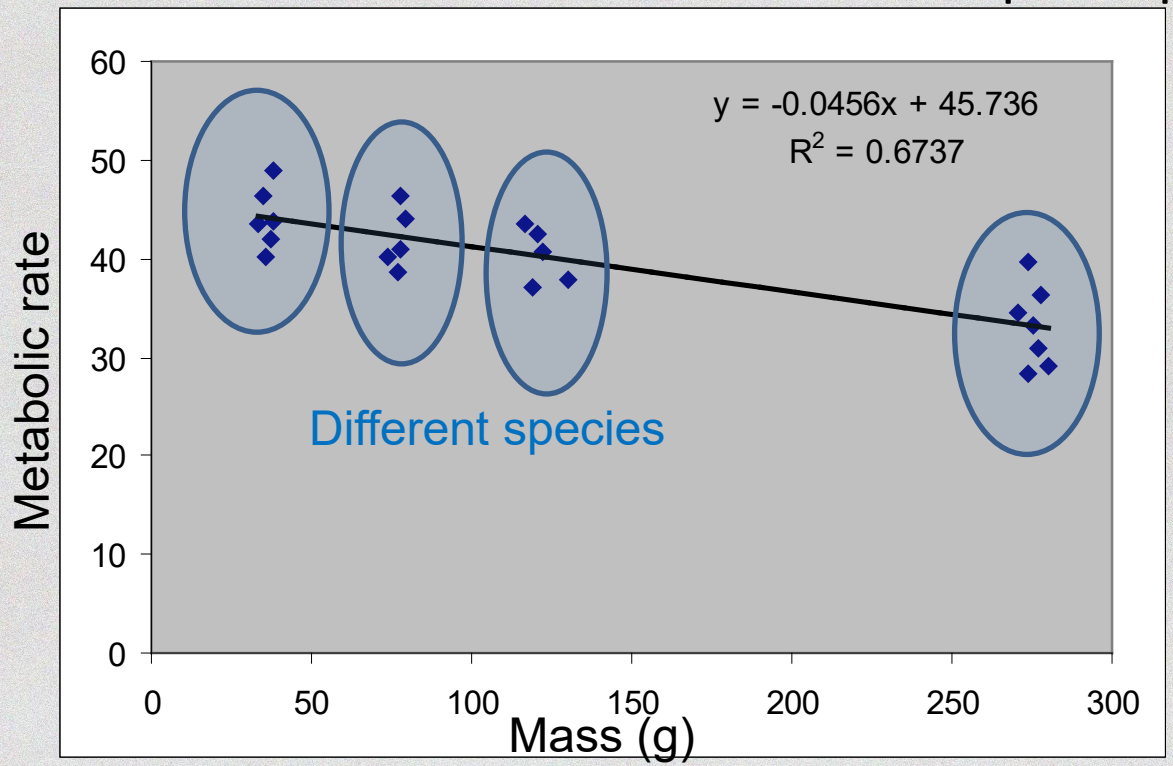
- Regression

We ignore phylogeny for now

habitat	species	metabolic rate	mass
desert	A	28.2	274
desert	A	33.2	275
desert	A	36.4	278
desert	A	34.6	271
desert	A	29.1	280
desert	A	31	277
desert	B	39.6	274
desert	B	40.8	122
desert	B	37.9	130
desert	B	37.1	119
desert	B	43.6	117
desert	B	42.4	121
mesic	A	46.3	35
mesic	A	42.1	37
mesic	A	43.5	33
mesic	A	48.8	38
mesic	A	43.7	38
mesic	A	40.1	36
mesic	B	41	78
mesic	B	44.1	79
mesic	B	46.4	78
mesic	B	40.2	74
mesic	B	38.6	77

Regression analysis

We use regression here to see if there is a linear effect of the X variable on the Y variable. If so, then we need to remove this effect to compare species.



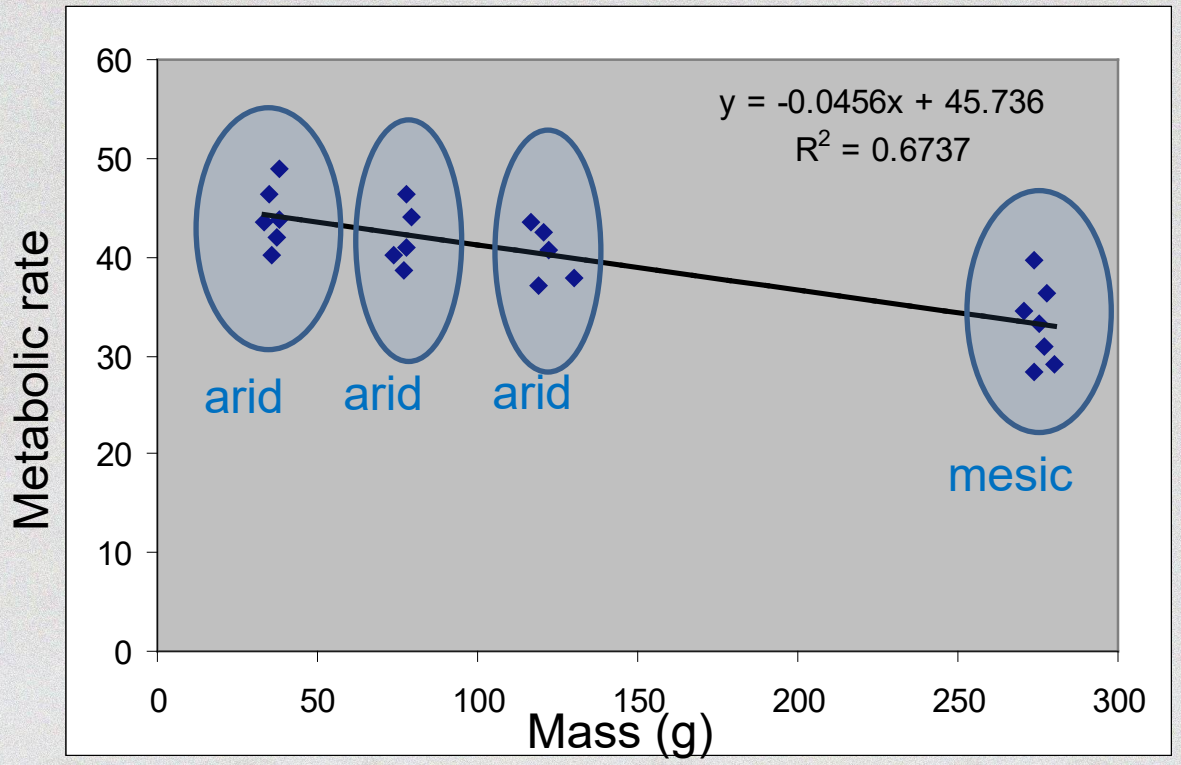
There is a significant mass effect between the 4 species
(but there might be no discernable mass effect within each species).

We should account for body mass when comparing these species.

Regression analysis

Might there be an mesic-arid difference?

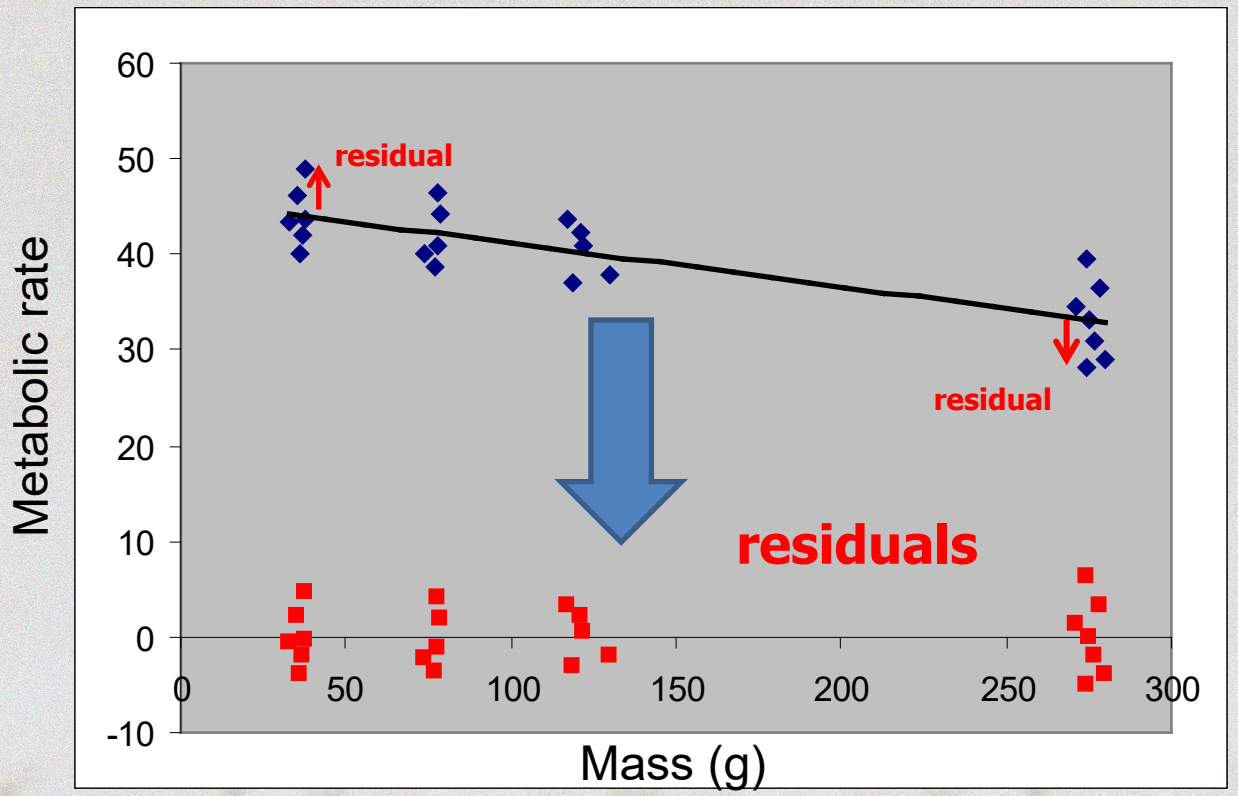
Is mesic still lower, if we account for the mass effect?



Regression residuals are really useful here

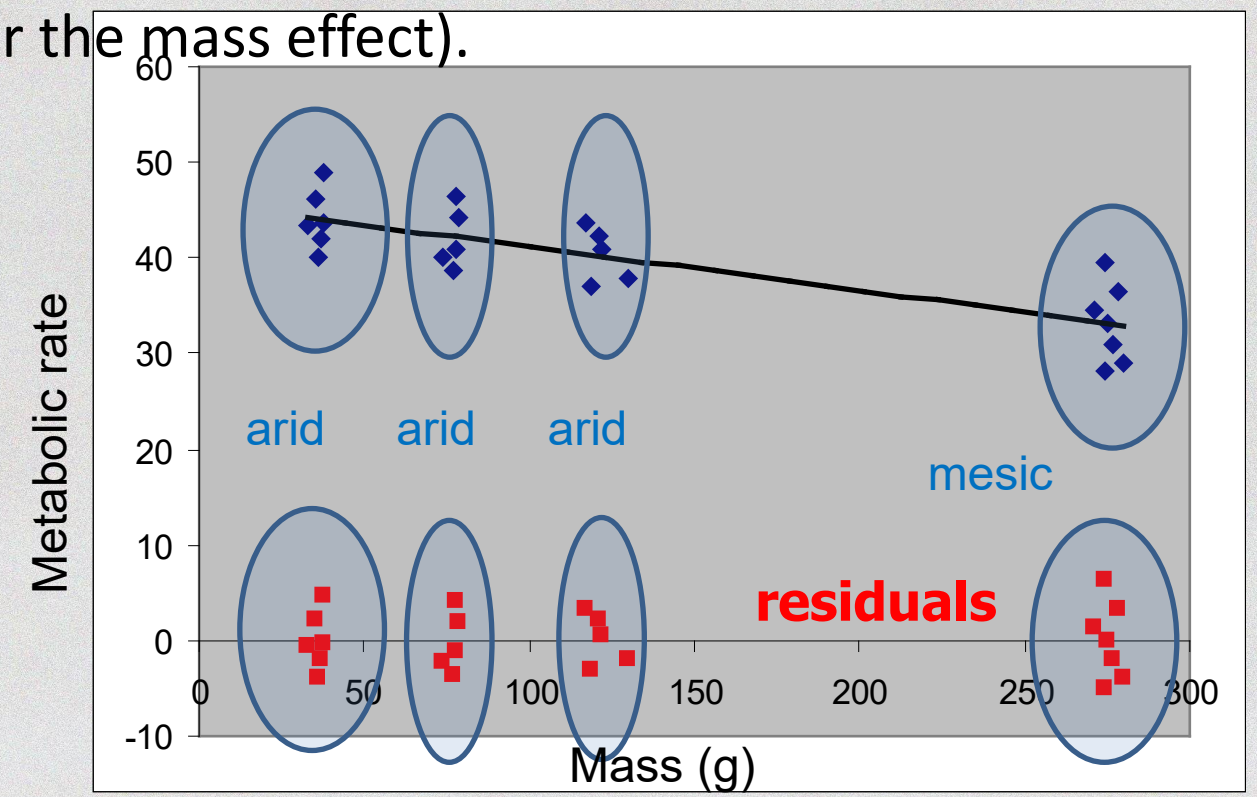
We can calculate residuals (how far a point is above or below predicted) from the regression line for each point.

Residuals remove any effect of X (e.g. mass) on Y (e.g. metabolic rate).



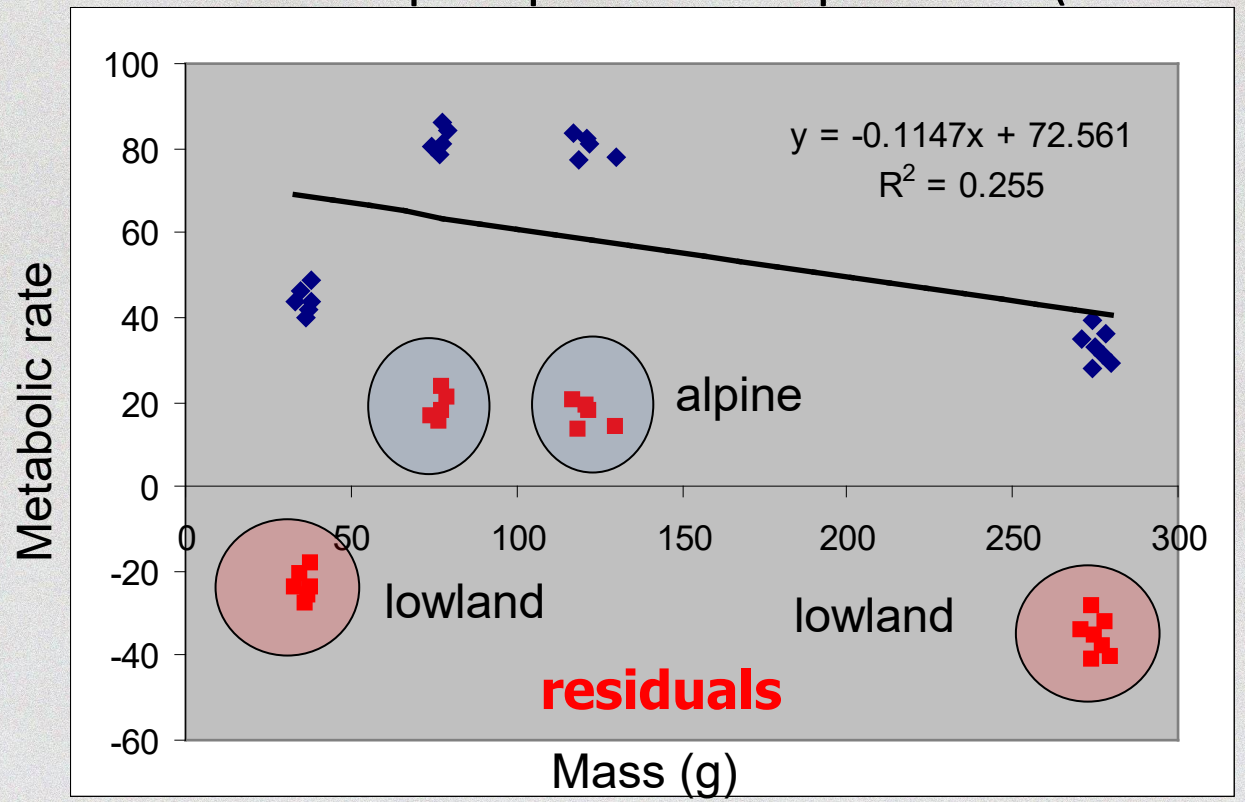
Desert-Mesic effect on regression residuals?

If we compare the residuals (by ANOVA), there are clearly no differences between arid and mesic species
(after accounting for the mass effect).



A different example of regression residuals in metabolic rate vs mass

In this example, the residuals clearly differ for the various species, showing that residuals can be a useful tool for multiple species comparisons (a bit like ANOVA).



(in this case, the overall regression has doubtful meaning)

We have been assuming “independence” of traits for multiple species

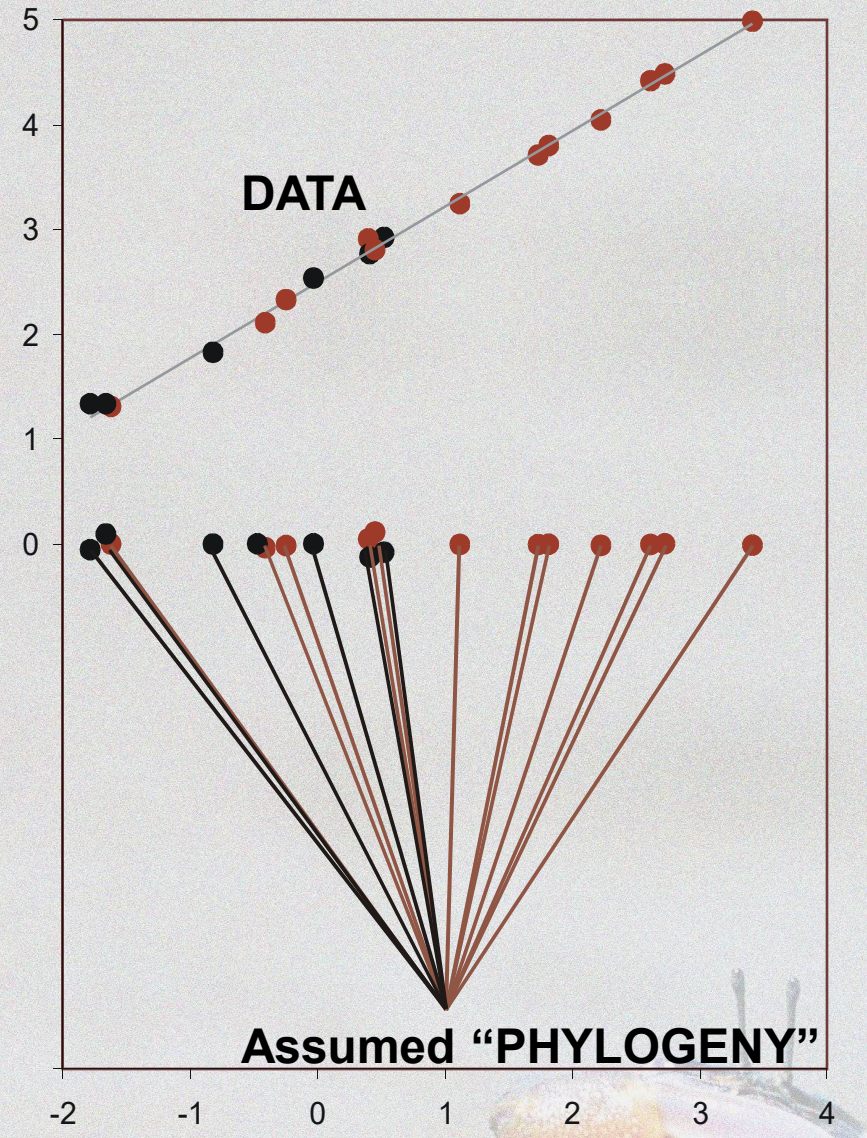
Consider this:

when analysing traits, an important question is whether the trait can be considered to have evolved “independently” in species (not whether the species are phylogenetically “independent”).

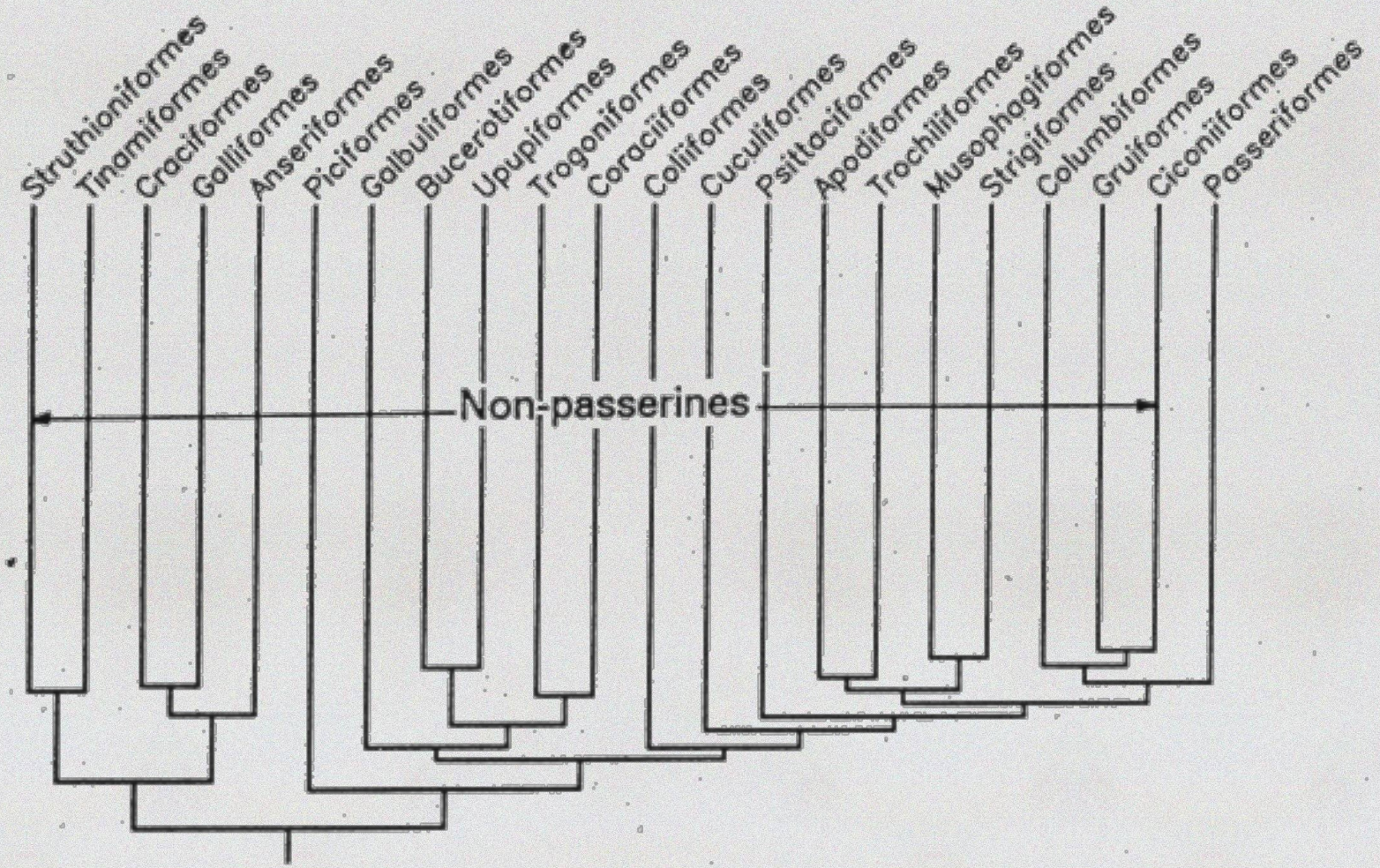
For example, with these species of birds (●) and mammals (●), if metabolic rate is “independent” of phylogeny then we can consider the DATA for these species to be independent

- In this case, the trait DATA are a “star” phylogeny

THIS MAY OR MAY NOT BE TRUE FOR DIFFERENT TRAITS



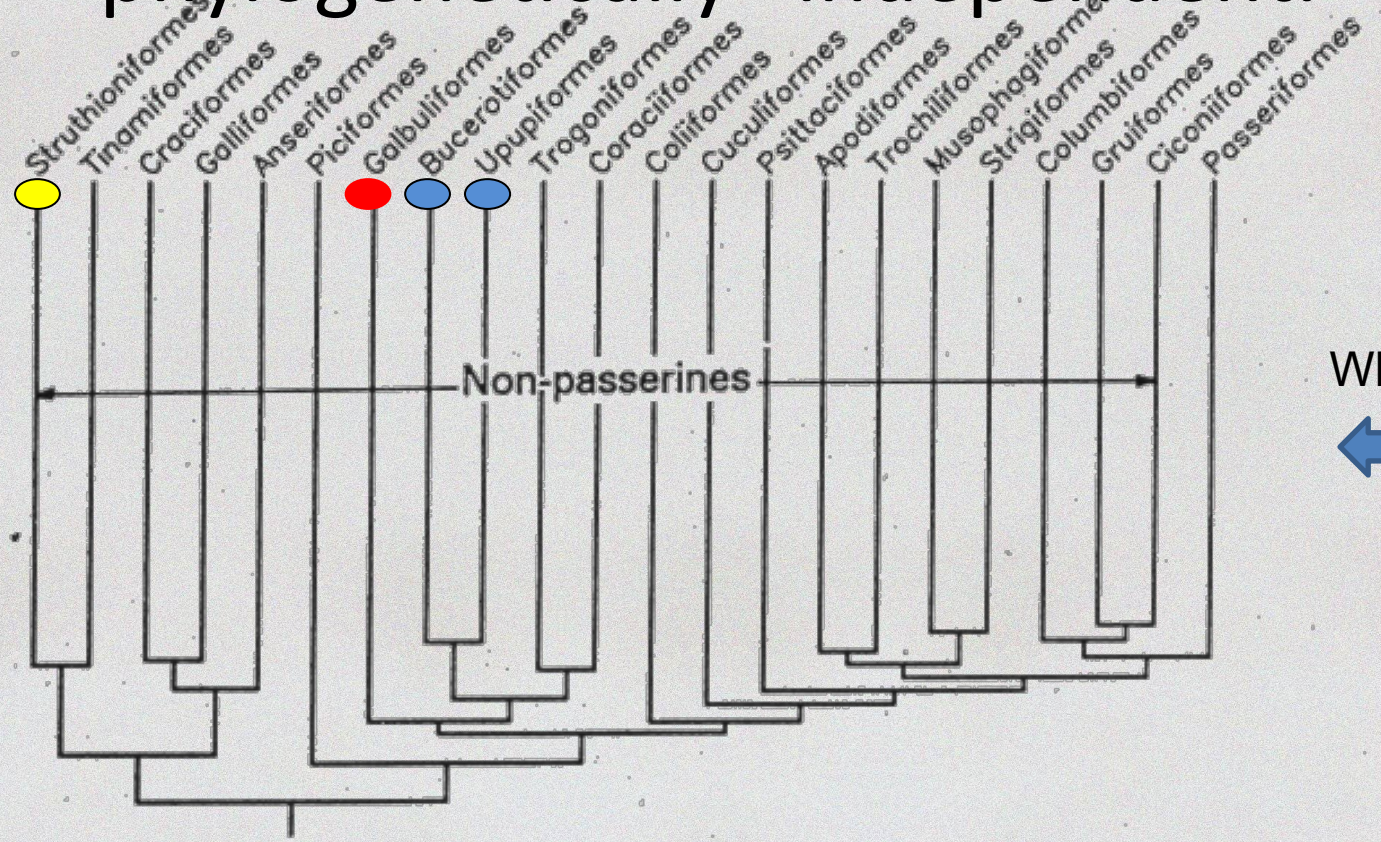
But actual phylogenies are NEVER a star!



So our data is NEVER independent!

What is the solution?

The statistical issue when analysing multiple species is the question: are the trait values for different species "phylogenetically" independent.



When did the trait evolve?

Clearly, ● are probably **NOT** phylogenetically "independent"

● and ● might be phylogenetically "independent"

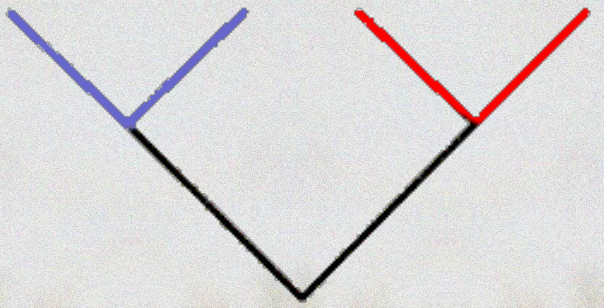
Independence is critical

Often, PHYLOGENY must be properly accounted for to reveal any real relationships

Treating dependent species as independent is a form of “pseudo-replication”

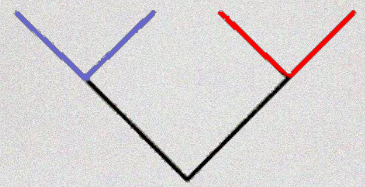
2 cocoon-forming *Neobatrachus* and 2 non-cocooning *Heleioporus*

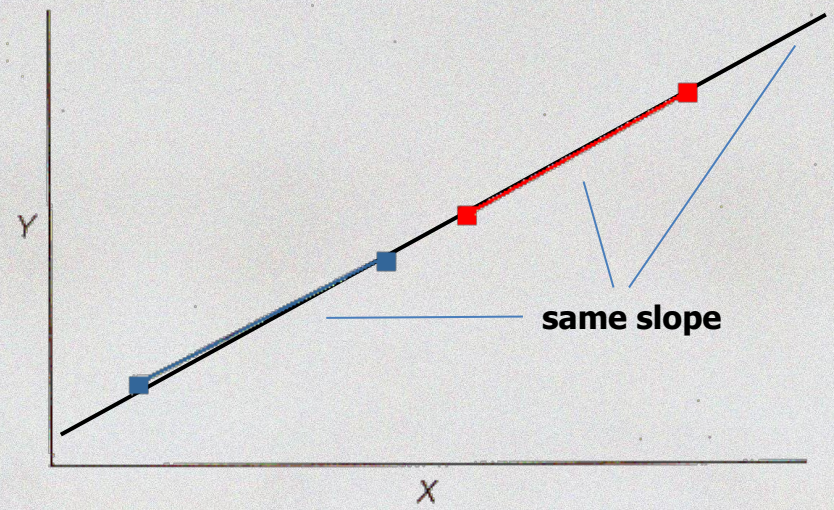
Four independent species ..... or ..... two independent samples?



.. if the species are phylogenetically “independent”

then the 4 species might conform to the same common regression relationship and can be considered to be independent

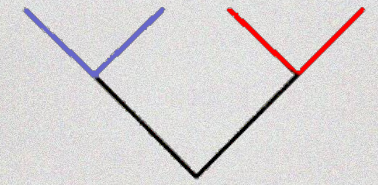


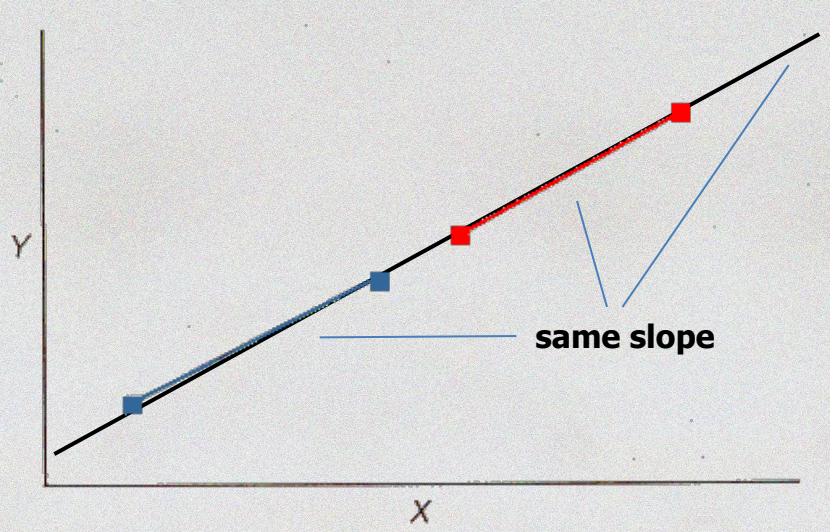


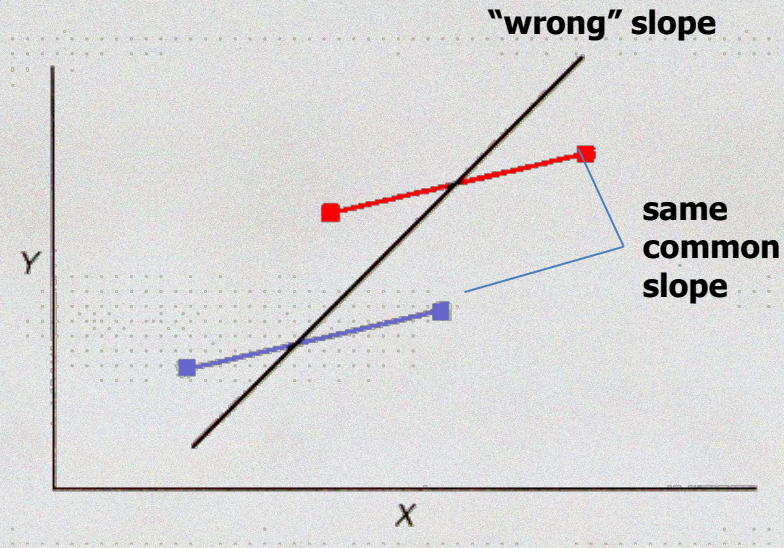
no phylogenetic effect

.. if the species are not phylogenetically “independent”

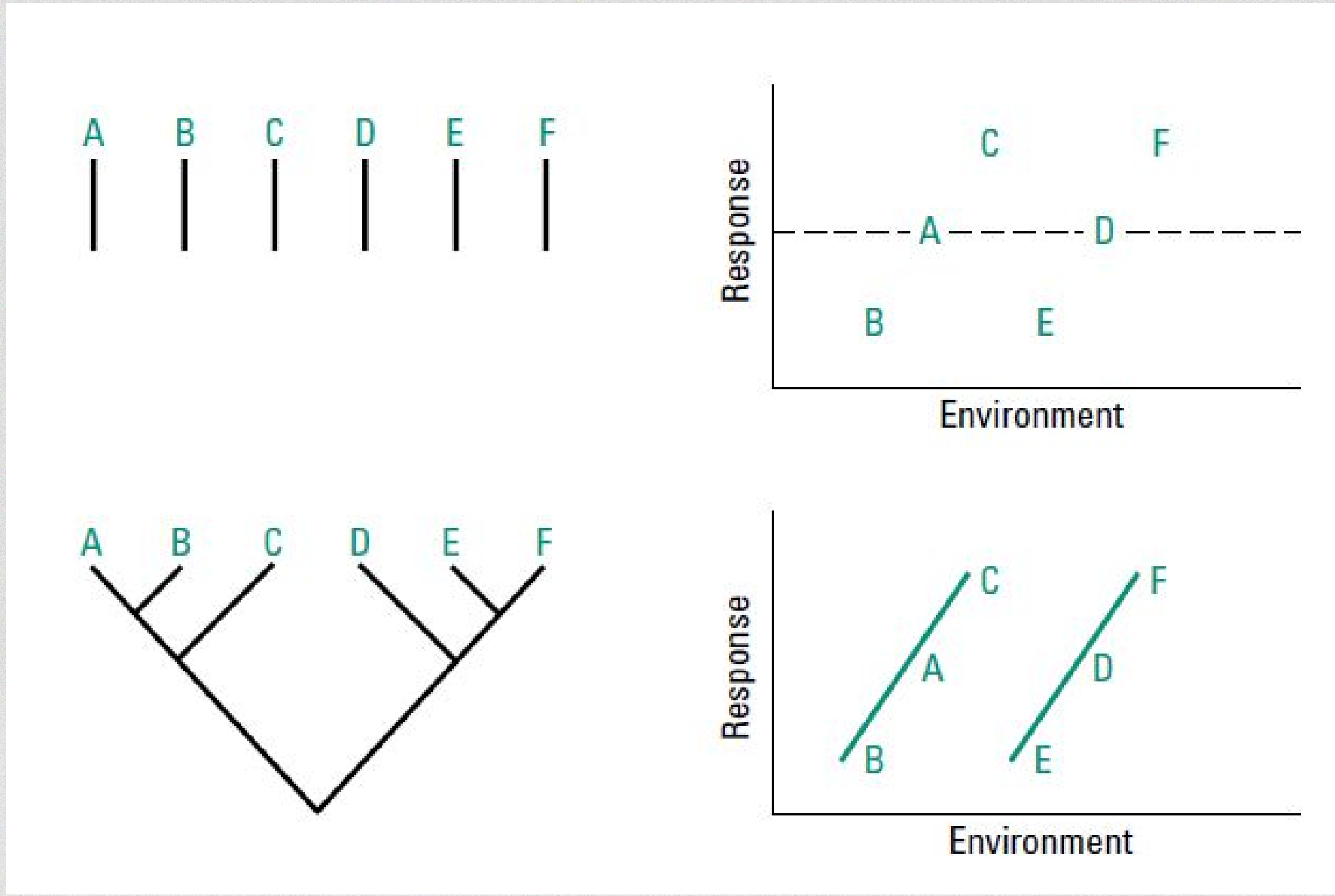
closely related pairs might conform to the same common slope but have an offset







no phylogenetic effect → **phylogenetic effect**



Willmer et al chapter 1 Fig. 1.3

Some METHODS for phylogenetic analysis

Taxonomic base:

- **analysis of covariance**
- **nested analysis of variance**

Phylogenetic base:

- **independent contrasts**
- **autoregression**
- **PVR**
- **PGLS**

PGLS is one of the most commonly used methods and allows us to separate the effects of phylogeny from the effects of other traits in question.